CLAIM AMENDMENTS

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (previously presented) A method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) providing an array comprising:

i) a substrate with a surface comprising discrete sites;

ii) a population of microspheres comprising at least first and second subpopulations, distributed at discrete sites on a surface of a substrate; and

iii) an enzyme attached at said discrete sites, wherein said enzyme is used to generate a signal from pyrophosphate;

b) providing a first hybridization complex comprising said first domain of a first target sequence and a first sequence primer, wherein said first hybridization complex is attached to said first subpopulation;

c) providing second hybridization complex comprising said second domain of a second target sequence and a second sequence primer, wherein said second hybridization complex is attached to said second subpopulation;

d) simultaneously extending said first and second primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primers, respectively;

e) detecting the release of pyrophosphate (PPi) with said enzyme attached at said discrete site within a common reaction chamber of said simultaneous extensions to determine the type of said first nucleotide added onto said first and second primers, respectively; and

f) determining sequences for said plurality of target nucleic acids.

2. (previously presented) A method according to claim 1 wherein at least said first hybridization complex is covalently attached to said first microsphere.

3. (previously presented) A method according to claim 1 wherein at least said first sequence primer is attached to said first microsphere.

4. (previously presented) A method according to claim 1 wherein said first and second hybridization complexes comprise:

a) said first and second target sequences;

b) said first and second sequence primers;

c) first and second capture probes, wherein said capture probes are covalently attached to said first and second microspheres, respectively.

5. (previously presented) A method according to claim 1, wherein said first and second hybridization complexes comprise said first and second target sequences, respectively, said first and second sequence primers, a first and second adapter probe, respectively, and first and second capture probes, respectively, covalently attached to said first and second microspheres.

6. (previously presented) A method according to claim 1 further comprising:

d) extending said first and second extended primers by the addition of a second nucleotide to the second detection position using said first enzyme; and

e) detecting the release of pyrophosphate (PPi) to determine the type of said second nucleotide added onto said first and second primers, respectively.

7. (previously presented) The method according to claim 1 wherein said PPi is detected by a method comprising:

a) contacting said PPi with a second enzyme that converts said PPi into ATP; and

b) detecting said ATP using a third enzyme, wherein said enzyme attached at said discrete sites comprises said second enzyme or said third enzyme.

8. (original) A method according to claim 7 wherein said second enzyme is sulfurylase.

9. (original) A method according to claim 7 wherein said third enzyme is luciferase.

10. (previously presented) A method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) providing first hybridization complex comprising a first target sequence and a first sequencing primer that will hybridize to the first domain of said first target sequence,

b) providing a second hybridization complex comprising a second target sequence and a second sequencing primer that will hybridize to the second domain of said second target sequence, wherein said first and second sequencing primers are covalently attached to microspheres distributed at discrete sites on a surface of a substrate, said discrete sites having an attached enzyme used to generate a signal from pyrophosphate;

c) determining the identity of a plurality of bases at said target positions, wherein said determining comprises simultaneously extending said first and second sequencing primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primers, respectively; and

d) detecting the release of pyrophosphate (PPi) with said enzyme attached at said discrete sites within a common reaction chamber of said simultaneous extensions to determine the type of said first nucleotide added onto said first and second sequencing primers, respectively.

11. (previously presented) A method according to claim 10 wherein said first hybridization complex and said second hybridization complex each comprise a capture probe.

12. (previously presented) A method according to claim 10 wherein said capture probe is a sequencing primer.

13. (previously presented) A method according to claim 10 wherein said determining comprises:

a) providing a sequencing primer hybridized to said second domain;

b) extending said primer by the addition of a first nucleotide to a first detection position using a first enzyme to form an extended primer;

c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer;

d) extending said primer by the addition of a second nucleotide to a second detection position using said enzyme; and

e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer.

14. (previously presented) The method according to claim 13 wherein said PPi is detected by a method comprising:

a) contacting said PPi with a second enzyme that converts said PPi into ATP; and

b) detecting said ATP using a third enzyme, wherein said enzyme attached at said discrete sites comprises said second enzyme or said third enzyme.

15. (original) A method according to claim 14 wherein said second enzyme is sulfurylase.

16. (original) A method according to claim 14 wherein said third enzyme is luciferase.

17. (previously presented) A method according to claim 10 wherein said determining comprises:

a) providing a sequence primer hybridized to said second domain;

b) extending said primer by the addition of a first protected nucleotide using a first enzyme to form an extended primer;

c) determining the identification of said first protected nucleotide;

d) removing the protection group;

e) adding a second protected nucleotide using said first enzyme; and

f) determining the identification of said second protected nucleotide.

18. (previously presented) A kit for nucleic acid sequencing comprising:

a) a composition comprising:

i) a substrate with a surface comprising discrete sites;

ii) a population of microspheres distributed on said sites; wherein said microspheres comprise different capture probes, wherein said array is configured for simultaneous contact of said different capture probes with a common reaction chamber; and

iii) an enzyme attached at said discrete sites, wherein said enzyme is used to generate a signal from pyrophosphate;

b) a first extension enzyme; and

c) dNTPs.

19. (previously presented)A kit according to claim 18 further comprising:

d) a second enzyme for the conversion of pyrophosphate (PPi) to ATP; and

e) a third enzyme for the detection of ATP, wherein said enzyme attached at said discrete sites comprises said second enzyme or said third enzyme.

20. (original) A kit according to claim 18 wherein said dNTPs are labeled.

21. (original) A kit according to claim 20 wherein each dNTP comprises a different label.

22. (previously presented) The method according to claim 1, wherein said substrate comprises discrete sites and said first and second subpopulations of microspheres are randomly distributed on said sites.

23. (previously presented) The method according to claim 22, wherein said discrete sites are wells, and said first and second subpopulations of microspheres are randomly distributed in said wells.

24. (previously presented) The method according to claim 10, wherein said substrate comprises discrete sites and said microspheres are randomly distributed on said sites.

25. (previously presented) The method according to claim 10, wherein discrete sites are wells, and said microspheres are randomly distributed in said wells.

26. (previously presented) The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate comprises a fiber optic bundle.

27. (previously presented) The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is selected from the group consisting of glass and plastic.

28. (currently amended) The kit according to claim 18, wherein said discrete sites are wells.

29. (previously presented) The kit according to claim 18 or 28, wherein said substrate is a fiber optic bundle.

30. (previously presented) The kit according to claim 18 or 28, wherein said substrate is selected from the group consisting of glass and plastic.

31. (previously presented) The method according to claim 1, wherein said microsphere array is decoded prior to providing first and second hybridization complexes.

32. (previously presented) The method according to claim 31, wherein said microspheres further comprise an identifier binding ligand that will bind a decoder binding ligand such that the identity and location of each microsphere can be determined.

33. (previously presented) A method according to claim 11 wherein said first hybridization complex and said second hybridization complex further comprise an adapter probe.

34. (previously presented) A method of sequencing a genome comprising:

a) amplifying a genome, thereby obtaining a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions;

b) providing an array comprising:

i) a substrate with a surface comprising discrete sites;

ii) a population of microspheres comprising at least a first and second subpopulation, distributed at said discrete sites; and

iii) an enzyme attached at said discrete sites, wherein said enzyme is used to generate a signal from pyrophosphate;

c) hybridizing sequencing primers to said first domains of said target sequences, wherein said hybridization complexes are attached to said microspheres;

d) simultaneously extending said primers by the addition of a first nucleotide to a first detection position using a first enzyme to form an extended primer; and

e) detecting the release of pyrophosphate (PPi) with said enzyme attached at said discrete sites within a common reaction chamber of said simultaneous extensions to determine the type of said first nucleotide added onto said primers; and

f) determining sequences for said genome.

35. (previously presented) The method of claim 1, wherein said enzyme attached at said discrete sites is attached to a microsphere.

36. (previously presented) The method of claim 1, wherein said first and second target nucleic acids comprise PCR amplification products.

37. (previously presented) The method of claim 1, wherein said first and second target nucleic acids comprise genomic DNA.

38. (previously presented) The method of claim 10, wherein said enzyme attached at said discrete sites is attached to a microsphere.

39. cancelled.

40. (previously presented) The method of claim 10, wherein said target sequences are covalently attached to said microspheres.

41. (previously presented) The method of claim 10, wherein said first and second target nucleic acids comprise PCR amplification products.

42. (previously presented) The method of claim 10, wherein said first and second target nucleic acids comprise genomic DNA.

43. (previously presented) The kit of claim 18, wherein said enzyme attached at said discrete sites is attached to a microsphere.

44. (new) The method of claim 1, wherein said common reaction chamber comprises a flow cell.

45. (new) The method of claim 44, further comprising washing unreacted nucleotides from said flow cell following said simultaneously extending said first and second primers.

46. (new) The method of claim 10, wherein said common reaction chamber comprises a flow cell.

47. (new) The method of claim 46, further comprising washing unreacted nucleotides from said flow cell following said simultaneously extending said first and second sequencing primers.

48. (new) The kit of claim 18, wherein said common reaction chamber comprises a flow cell.

49. (new) The method of claim 34, wherein said common reaction chamber comprises a flow cell.

50. (new) The method of claim 49, further comprising washing unreacted nucleotides from said flow cell following said simultaneously extending said primers.